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An Italian retrospective study on the routine clinical use of low-dose alemtuzumab in relapsed/refractory chronic lymphocytic leukaemia patients

Agostino Cortelezzi,[1,2] Giuseppe Gritti,[2] Luca Laurenti,[3] Antonio Cuneo,[4] Stefania Ciolli,[5] Nicola Di Renzo,[6] Pellegrino Musto,[7] Francesca R. Mauro,[8] Nicola Cascavilla,[9] Lorenzo Falchi,[10] Francesco Zallio,[11] Vincenzo Callea,[12] Francesco Maura,[1] Sara Martinelli,[4] Alfonso Piciocchi,[13] Gianluigi Reda[1], and Robin Foà[8] on behalf of the GIMEMA Chronic Lymphoproliferative Disorders Working Party

[1]Haematology-BMT Unit, IRCCS Ca'Granda Ospedale Maggiore Policlinico Foundation, Milan, [2]Department of Medical Sciences, University of Milan, Milan, [3]Institute of Haematology, 'Cattolica' University, Rome, [4]Institute of Haematology, University of Ferrara, Ferrara, [5]SODc of Haematology, 'Careggi' University Hospital, Firenze, [6]Haematology Unit, 'Vito Fazzi' Hospital, Lecce, [7]Department of Haemato-oncology, IRCCS Centro di Riferimento Oncologico di Basilicata, Rionero in Vulture, [8]Haematology, Department of Cellular Biotechnology and Haematology, 'Sapienza' University, Rome, [9]Haematology Unit, IRCCS 'Casa Sollievo della Sofferenza' Hospital, San Giovanni Rotondo (FG), Italy, [10]Department of Haemato-oncology, University of Perugia and 'S. Maria' Hospital, Terni, [11]Haematology Unit, 'SS Antonio e Biagio e C. Arrigo' Hospital, Alessandria, [12]Haematology Unit, 'Bianchi-Melacrinò-Morelli' Hospital, Reggio Calabria, and [13]GIMEMA Data Centre, Rome, Italy

Summary

Low-dose alemtuzumab has shown a favourable toxicity profile coupled with good results in terms of efficacy in relapsed/refractory chronic lymphocytic leukaemia (CLL). We conducted a multicentre retrospective study on the routine clinical use of low-dose alemtuzumab in this patient setting. One hundred and eight relapsed/refractory CLL patients from 11 Italian centres were included in the analysis. All patients had an Eastern Cooperative Oncology Group performance status ≤2 and the majority (84%) had adenopathies <5cm. Low-dose alemtuzumab was defined as a total weekly dose ≤45 mg and a cumulative dose ≤600 mg given for up to 18 weeks. The overall response rate was 56% (22% complete remissions). After a median follow-up of 42·2 months, the median overall survival and progression-free survival were 39·0 and 19·4 months, respectively. In univariate analysis, response was inversely associated with lymph node ($P = 0{\cdot}01$) and spleen ($P = 0{\cdot}02$) size, fludarabine-refractoriness ($P = 0{\cdot}01$) and del(11q) ($P = 0{\cdot}009$). Advanced age and del(17p) were not associated with a worse outcome. Cumulative dose of alemtuzumab was not associated to response. Toxicities were usually mild and manageable; severe infections occurred in seven patients (7%) during therapy. This retrospective analysis confirms that low-dose alemtuzumab is a valid and currently used therapeutic option for the treatment of relapsed/refractory CLL.

Keywords: alemtuzumab, chronic lymphocytic leukaemia, infection, monoclonal antibodies.

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Correspondence: Agostino Cortelezzi, MD, UO Ematologia-CTMO, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Dipartimento di Scienze Mediche, Università degli Studi di Milano, Via F. Sforza 35, 20122 Milano, Italy.
E-mail: agostino.cortelezzi@unimi.it

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Introduction

Alemtuzumab (Campath-1H), is an IgG1 kappa monoclonal antibody specific for the cell surface glycoprotein CD52 expressed on normal and malignant B and T lymphocytes (Gribben & Hallek, 2009; Hallek & Pflug, 2011). Over the last decade, alemtuzumab as single agent has been utilized in chronic lymphocytic leukaemia (CLL) as first-line treatment (Osterborg *et al*, 1996; Lundin *et al*, 2002; Hillmen *et al*, 2007) and in relapsed/refractory patients (Osterborg *et al*, 1997; Keating *et al*, 2002; Rai *et al*, 2002; Stilgenbauer & Dohner, 2002; Lozanski *et al*, 2004; Stilgenbauer *et al*, 2009). Results have been encouraging, because the antibody has shown activity even when the commonly used fludarabine-based chemoimmunotherapy strategies have failed, at least in patients without bulky disease (Keating *et al*, 2002; Rai *et al*, 2002; Lozanski *et al*, 2004; Stilgenbauer *et al*, 2009). Moreover, alemtuzumab appears to be active on CLL cells in a p53 independent manner, thus being an effective therapy for patients carrying del(17p) or mutations of the *TP53* gene, that are commonly resistant to conventional treatments (Stilgenbauer & Dohner, 2002; Lozanski *et al*, 2004; Zenz *et al*, 2009).

However, the major obstacle to the widespread use of alemtuzumab outside clinical trials is that treatment is associated with profound immunosuppression leading to a high rate of severe infections (Keating *et al*, 2002; Nosari *et al*, 2004; Stilgenbauer *et al*, 2009). The expected rate of severe complications associated with alemtuzumab therapy has hampered the use of this monoclonal antibody in patients categorized as frail by clinicians (Nosari *et al*, 2004, 2008; Morrison, 2010).

It has been previously shown that alemtuzumab, given at a lower dose, is a safe and effective treatment in CLL (Cortelezzi *et al*, 2005, 2009; Laurenti *et al*, 2005; Bezares *et al*, 2011; Gritti *et al*, 2011). Alemtuzumab was administered at a weekly dose that was one-third (30 vs. 90 mg) and a cumulative dose that was half (540 vs. 1080 mg) of those commonly employed (Cortelezzi *et al*, 2005, 2009; Gritti *et al*, 2011), with a prolonged treatment course (18 vs. 12 weeks). The safety results of these studies have shown that severe infections occurred in about 8% of patients without deaths during treatment; this incidence appears lower than those reported when alemtuzumab is administered at conventional doses (Keating *et al*, 2002; Cortelezzi *et al*, 2009; Stilgenbauer *et al*, 2009; Gritti *et al*, 2011).

The perception that alemtuzumab may be administered in a safer way using lower doses came into clinical practice in several Italian haematological institutions far before the definitive results of the abovementioned trials were available. In the present retrospective study, we have therefore evaluated the routine clinical use of low-dose alemtuzumab in 11 Italian haematological centres.

Patients and methods

Patients

Adult (≥18 years) CLL patients treated with low-dose alemtuzumab outside clinical trials were included in this retrospective study. All the participating centres adopted the policy to use alemtuzumab at low dose. Alemtuzumab was defined at low-dose if the total weekly dose was ≤45 mg and the cumulative dose was ≤600 mg. Duration of treatment was accepted up to 18 weeks of therapy; both subcutaneous (SC) and intravenous (IV) administrations were allowed. Other inclusion criteria were diagnosis of CLL requiring treatment according to the 1996 National Cancer Institute-sponsored Working Group (NCIWG) guidelines (Cheson *et al*, 1996) and at least one previous therapy not containing alemtuzumab as single agent or in combination.

The study was conducted in accordance with the Declaration of Helsinki, approved by the Ethic Committee and registered at the regulatory bureau (Agenzia Italiana del Farmaco) in accordance with Italian law.

Assessment

A set of clinical variables were documented at baseline, including previous treatments, age, sex, lymph node size, presence of hepato-splenomegaly, spleen size evaluated by imaging, Binet stage and haematological parameters. A series of biological prognostic markers were collected, including the analysis of genomic aberrations by interphase fluorescence *in situ* hybridization (FISH) for del(17p13), del(11q22), trisomy 12 and del(13q14); *IGHV* mutation status by sequencing; CD38 and ZAP70 expression. Positivity for CD38 and ZAP70 was defined according to local laboratory practice.

Treatment schedule, planned and given doses were assessed. Response was defined according to the NCIWG criteria (Cheson *et al*, 1996). Infusion-related toxicities and infections were documented and reported according to the NCI CTCAE v3.0 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf). Haematological toxicity was assessed by means of the NCIWG guidelines (Cheson *et al*, 1996).

Statistical considerations

Differences in the distributions of prognostic factors in subgroups were analysed by chi-square or the Fisher's exact test, and by the Kruskal–Wallis test. The probability of overall survival (OS) and progression-free survival (PFS) was estimated using the Kaplan–Meier method. The log-rank test was used to compare treatment effect and risk factor categories, while 95% confidence intervals (95% CIs) were estimated using the Simon and Lee method. Logistic regression and Cox proportional hazard regression models were performed to

examine risk factors affecting treatment response [responders: complete remission (CR), partial remission (PR); non-responders: stable disease (SD), progressive disease (PD)] and survival outcomes. All tests were two-sided, accepting $P \leq 0{\cdot}05$ as indicating a statistically significant difference. All statistical analyses were performed with the statistical software environment R (R Development Core Team, 2009).

Results

Patients characteristics

One hundred and eight CLL patients, treated between March 2002 and December 2009 at 11 Italian centres, were included in the analysis. The baseline patient characteristics are summarized in Table I. Median age was 68 years (range 43–85) and 45 patients (42%) were older than 70 years. The majority of patients had advanced disease; almost half of them were Binet stage C ($n = 50$, 46%). Seventeen patients (16%) presented with bulky lymph nodes (≥5 cm) and 59 (55%) had a palpable splenomegaly. Performance status (PS) according to the Eastern Cooperative Oncology Group (ECOG) was 0–1 in 94 patients (87%) and two in 14 patients (13%). No patient presented with a poorer performance status. Patients with an ECOG PS of two were characterized by advanced disease (79% Binet stage C), bulky adenopathies (36%) and fludarabine refractoriness (79%). The median number of previous lines of therapy was two (range 1–6). Most patients were treated with chlorambucil ($n = 81$, 75%); 36 (33%) patients were treated with single-agent fludarabine, 35 were exposed to rituximab-based chemoimmunotherapy (32%) and 36 (33%) were treated with fludarabine in combination with cyclophosphamide, with or without rituximab. Refractoriness to alkylating agents and fludarabine-containing regimens was documented in 59 (55%) and 48 (44%) patients, respectively.

The biological risk profile was available only for some of the cohort (see Table I). A high proportion of patients showed an unfavourable profile: 44% were positive for del(17p) or del(11q) at FISH analysis; an unmutated *IGHV* profile was detected in 67% of cases; CD38 and ZAP70 positivity was present in 51% and 60% of cases, respectively.

Table I. Baseline demographic and clinical characteristics.

	Number of patients (%) or median (range)
Age (years)	68 (43–85)
≥70	45 (42)
≥65	72 (67)
Sex	
Male	73 (68)
Female	35 (32)
Binet stage	
A	20 (19)
B	38 (35)
C	50 (46)
Time since initial diagnosis (years)	4·9 (0·7–23·0)
Prior lines of therapy	2 (1–6)
Previous treatment	
Chlorambucil	81 (75)
Fludarabine single agent	36 (33)
Rituximab + chemotherapy	35 (32)
(R)-FC	36 (33)
Autotransplant	2 (2)
Alkylating agents refractory	59 (55)
Fludarabine refractory	48 (44)
Maximum lymph node size (cm)	2·5 (0·5–13·5)
≥5 cm	17 (16)
Palpable hepatomegaly	43 (40)
Palpable splenomegaly	59 (55)
Spleen size in cm, evaluated by imaging ($n = 85$)	14 (9–25)
>12 cm	58 (68)
ECOG PS	
0–1	94 (87)
2	14 (13)
White blood cell count ($\times 10^9$/l)	54·9 (2·1–288)
Haemoglobin concentration (g/l)	119 (60–151)
Platelet count ($\times 10^6$/l)	47 (9–272)
Hierarchical FISH subgroups ($n = 97$)	
del(17p)	25 (26)
del(11q)	18 (19)
Trisomy 12 (no 11q- or 17p-)	8 (8)
Normal	33 (34)
Isolated del(13q)	17 (18)
IGHV mutation status ($n = 49$)	
Mutated	16 (33)
Unmutated	33 (67)
CD38 expression ($n = 92$)	47 (51)
ZAP70 expression ($n = 80$)	48 (60)

(R)-FC, fludarabine (F) plus cyclophosphamide (C) with or without rituximab (R); ECOG PS, Eastern Cooperative Oncology Group performance status; FISH, fluorescence *in situ* hybridization.

Schedule

Four schedules of low-dose alemtuzumab were reported. Fifty-five patients (51%) received 10 mg of alemtuzumab infusion three times weekly; the planned treatment duration was 10 weeks in 17 patients, 12 weeks in three and 18 weeks in 35 patients. A second cohort of 37 patients (34%) started therapy according to the previously described schedule and, when the lymphocyte count was reduced by 1 log, they were put on a weekly infusion of 30 mg up to 18 weeks of therapy. Eleven patients (10%) received 30 mg of alemtuzumab once weekly for 12 weeks ($n = 6$) or 18 weeks ($n = 5$). In the remaining five cases (5%), 15 mg of alemtuzumab was administered thrice weekly for 12 weeks. In 14 cases (13%), alemtuzumab was administered by intravenous (IV) infusion, while the remaining patients were treated subcutaneously (SC). Three patients were concomitantly treated with oral dexamethasone at a dose of 0·75–1 mg/d.

Treatment was completed in 60 patients (55%), and 14 additional patients received more than 75% of the planned therapy. Only 17 patients (16%) received <50% of the planned treatment, four of whom (4%) stopped treatment before completing the fourth week of therapy.

Efficacy

Response Most patients (n = 97, 90%) were assessed by means of imaging (computerized tomography scan or echography) and/or bone marrow examination. Only one patient was merely evaluated clinically; the remaining 10 patients showed disease progression during treatment.

The overall response rate (ORR) was 56%, with 24 patients (22%) achieving CR; stabilization and progression of disease were observed in 24 (22%) and 24 patients (22%), respectively (Table II). The univariate analysis for response is detailed in Table SI. Response rates in elderly patients (≥70 years) were not significantly different compared to those of younger patients [Odds Ratio (OR): 1·4, 95% CI: 0·6–2·9, P = 0·43]. There was an inverse association between lymph node size and response (P = 0·01); in particular, patients with bulky lymph nodes (≥5 cm) had an ORR of 29%, significantly lower compared to the 60% recorded in patients presenting without significant adenopathies (OR: 3·7, 95% CI: 1·2–11·4, P = 0·01). Similarly, spleen size negatively influenced response (OR: 0·9, 95% CI: 0·78–0·98, P = 0·02). When patients were stratified according to Binet stage, a trend towards a lower response with stage progression was observed, although this result was not significant (P = 0·07). None of the 14 patients with an ECOG PS of 2 responded to treatment (P < 0·0001).

There was an inverse correlation between the number of previous treatment lines and response (P = 0·0098). Patients with a fludarabine-refractory disease showed significantly poorer responses compared to the others (OR: 4·4, 95% CI: 1·3–14·5, P = 0·01).

Patients carrying del(11q) showed a poor response (OR: 4·5, 95% CCI: 1·5–13·9, P = 0·0061); this adverse outcome remained significant in the multivariate analysis that included the presence of bulky lymph nodes (P = 0·0348). The analysis of the other biological prognostic factors did not highlight any difference in response rates. In particular, patients carrying del(17p) showed a similar ORR compared to the other patients (OR: 0·8, 95% CCI: 0·3–2·1, P = 0·68).

Finally, no relationship between dose of alemtuzumab and response was observed both in the full cohort of patients and in the subgroups analysed (P = 0·39). Similarly, the schedule of administration did not appear to influence the response rates (P = 0·31).

Multivariate analysis for response was performed, including previous treatment with fludarabine, lines of treatment, age, white blood cell count, spleen size, bulky lymph nodes and del(11q). Spleen size (OR: 0·9, 95% CCI: 0·77–0·99, P= 0·03) and presence of del(11q) (OR: 5·5, 95% CCI: 1·3–22·7, P = 0·02), were identified as independent negative factors while presence of lymph nodes ≥5 cm (OR: 3·8, 95% CCI: 1·0–14·9, P = 0·06) showed a non-significant trend toward lower response.

Overall survival and progression-free survival After a median follow-up of 42·2 months (range 2·1–91·9), 55 deaths were recorded, 44 of which were related to CLL (disease progression, associated with lethal infections in most cases).

Table II. Responses by clinical and biological characteristics.

		n	ORR no. (%)	CR no. (%)	PR no. (%)	SD no. (%)	PD no. (%)	*P*
All		108	60 (56)	24 (22)	36 (34)	24 (22)	24 (22)	
FISH	17p-	25	15 (60)	6 (24)	9 (36)	8 (32)	2 (8)	0·68
	11q-	18	5 (28)	4 (22)	1 (6)	8 (44)	5 (28)	0·0061
	Other	58	37 (64)	15 (26)	22 (38)	7 (12)	14 (24)	
Age (years)	<70	63	37 (59)	15 (24)	22 (35)	12 (19)	14 (22)	0·43
	5 ≥70	45	23 (51)	9 (20)	14 (31)	12 (27)	10 (22)	
Binet stage	A	20	14 (70)	5 (25)	9 (45)	4 (20)	2 (10)	0·07
	B	38	24 (63)	11 (29)	13 (34)	6 (16)	8 (21)	
	C	50	22 (44)	8 (16)	14 (28)	14 (28)	14 (28)	
Maximum lymph node size (cm)	<5	81	49 (60)	21 (26)	28 (35)	17 (21)	15 (19)	0·01
	≥5	17	5 (29)	2 (12)	3 (18)	5 (29)	7 (41)	
ECOG PS	<2	94	60 (64)	24 (26)	36 (38)	17 (18)	17 (18)	<0·0001
	=2	14	0 (0)	0 (0)	0 (0)	7 (50)	7 (50)	
Previous treatment	Fludarabine-refractory	48	17 (35)	3 (6)	14 (29)	12 (25)	19 (40)	0·01
	Other	60	43 (72)	21 (35)	22 (37)	12 (20)	5 (8)	

FISH, fluorescence *in situ* hybridization; ECOG PS, Eastern Cooperative Oncology Group performance status; ORR, overall response rate; CR, complete remission; PR, partial remission; SD, stable disease; PD, progressive disease.

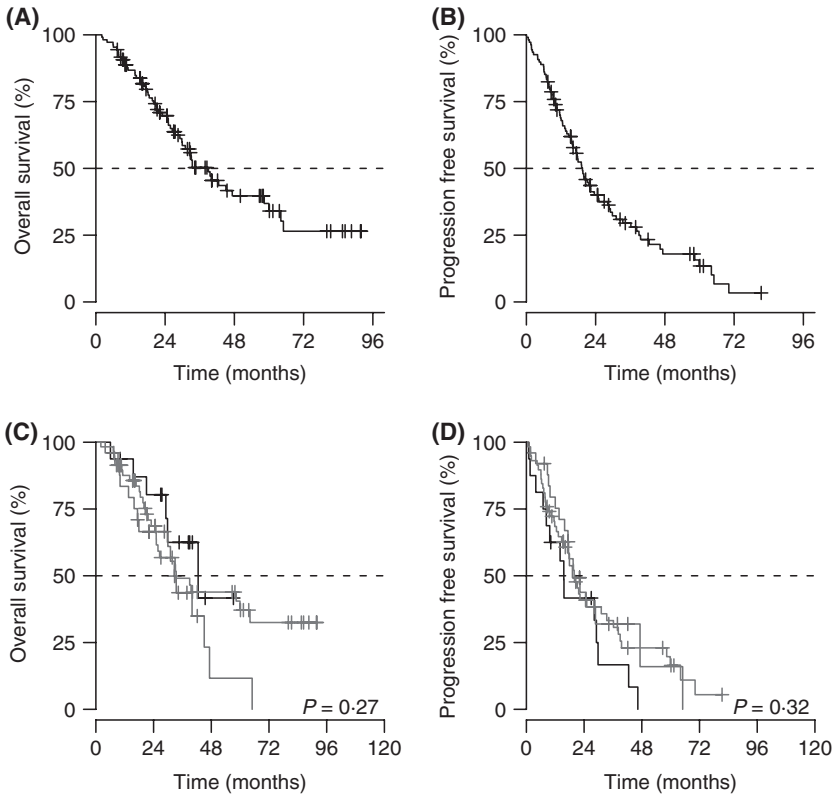

Fig 1. (A) Overall survival and (B) progression-free survival of the full patient series (n = 108). (C) Overall survival and (D) progression-free survival according to cytogenetic subgroup; red line: del(17p) (n = 25), black line: del(11q) (n = 18), green line: other (n = 58).

Causes of death unrelated to CLL included seven patients with second tumours and one patient deceased after a major trauma. In three cases, it was not possible to document the cause of death.

Median OS and PFS were 39·0 months (95% CI: 29·8–58·4) and 19·4 months (95% CI: 15·9–23·5), respectively (Fig 1A, B). Variables associated with a shorter OS were presence of bulky lymph nodes [29·8 months (95% CI: 13·6–33·4) vs. 45·1 months (95% CI: 32·6–64·1), P = 0·017, Fig 2A] and fludarabine-refractoriness [26·5 months (95% CI: 18·5–33·1) vs. not reached (95% CI: 33·0 – not reached), P = 0·003, Fig 2C]. PFS was significantly reduced in patients with bulky lymph nodes [13·6 months (95% CI: 3·9–19·0) vs. 21·2 months (95% CI: 16·6–28·7), P = 0·01, Fig 2B] and in fludarabine-refractory patients [12·5 months (95% CI: 9·2–17·3) vs. 20·0 (95% CI: 14·0 – not reached), P = 0·004, Fig 2D].

Del(11q) and/or del (17p) did not influence patient survival (Fig 1C). In particular, in patients carrying del(17p) showed no difference in OS (Fig 1C) and PFS (Fig 1D) compared to the other patients.

Advanced age (≥70 years) did not influence PFS (Fig S1A), while a significantly reduced OS was observed (P = 0·03, supplementary Fig 1A). Despite influencing treatment response, spleen size did not show any significant effect on OS and PFS (P = 0·47). Similarly, the treatment schedule did not influence patient outcome (P = 0·91, data not shown).

Safety

Alemtuzumab was well tolerated and no deaths were observed during therapy. A summary of adverse events (AEs) is shown in Table III. Injection-related events included fever (with or without chills and rigours) and injection site reactions, which occurred in 39% and 55% of the patients treated with SC alemtuzumab, respectively; the majority of events were mild (grade 1–2) and severe injection reactions and fever occurred in 3 (3%) and 2 (2%) patients, respectively. Grade 1–2 fever complicated the infusion of 6 patients (43%) treated with alemtuzumab IV; no severe infusion-related event was however reported.

Haematological toxicities were frequent, but usually mild; grade 3–4 neutropenia, thrombocytopenia and anaemia occurred in 29%, 6% and 6% of patients, respectively.

Primary prophylaxis with acyclovir or analogues was performed in all patients. Weekly cytomegalovirus (CMV) reactivation surveillance by means of early pp65 CMV antigenaemia or by CMV DNA quantification was carried out in all patients. CMV reactivation occurred in 38 patients (35%) and was managed with temporary treatment interruption. There was only one case of CMV disease, which resolved after treatment with intravenous ganciclovir.

Non-CMV infections were documented in 37 patients (34%). However, severe (grade 3–4) infections occurred only

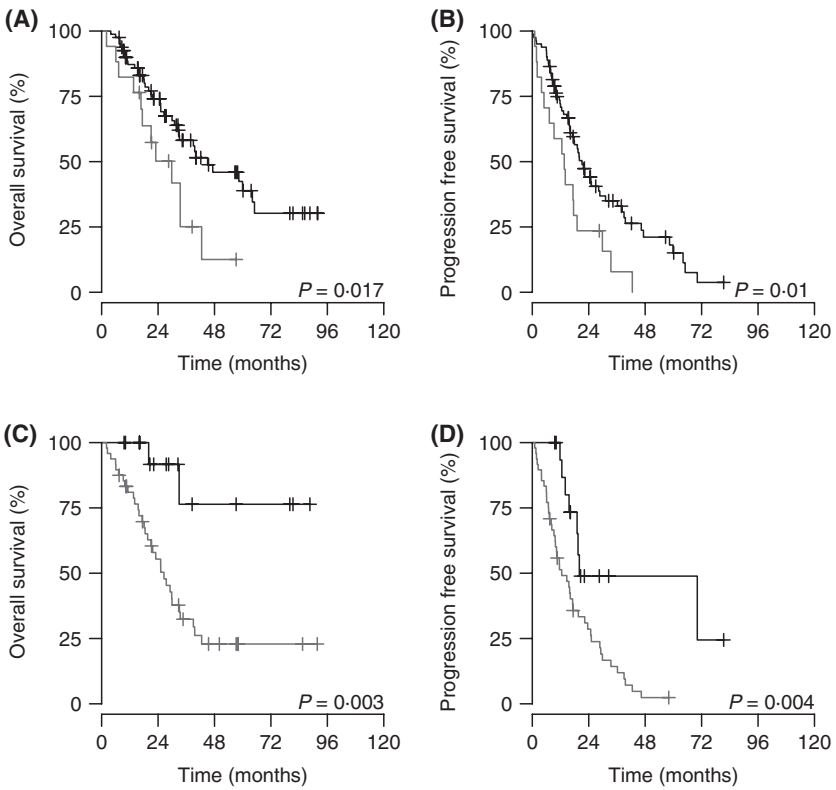

Fig 2. (A) Overall survival and (B) progression-free survival according to lymph node size; red line: lymph nodes ≥5 cm (n = 17), black line: lymph nodes <5 cm (n = 81). (C) Overall survival and (D) progression-free survival according to response to fludarabine; red line: fludarabine refractory (n = 48), black line: fludarabine sensitive or not exposed (n = 60).

Table III. Adverse events using the National Cancer Institute Toxicity Grades.

Patients experiencing adverse event (n = 108)			
	no. (%)		
	All Grade	Grade 1–2	Grade 3–4
Infusion related (SC only, n = 94)			
Injection site reaction	52 (55)	49 (52)	3 (3)
Fever	34 (36)	32 (34)	2 (2)
Haematological			
Anaemia	53 (49)	47 (44)	6 (6)
Neutropenia	64 (62)	34 (33)	30 (29)
Thrombocytopenia	41 (38)	35 (33)	6 (6)
Infections			
CMV events	38 (35)	37 (34)	1 (1)
Non-CMV infections	37 (34)	30 (28)	7 (7)

SC, subcutaneously; CMV, cytomegalovirus.

in seven patients (7%) with no difference between responders (n = 4) and non-responders (n = 3); they included bacterial pneumonia (n = 3), soft-tissue infection (n = 2), sepsis (n = 1) and fever of unknown origin (n = 1).

Serology for hepatitis B virus (HBV) was available in 83 patients (Table IV). Five patients started therapy with active HBV infection, defined by the presence of hepatitis B surface antigen and/or plasmatic HBV-DNA, and nine patients had a serological profile of occult infection, defined by the presence of antibodies to the hepatitis B core antigen. In these patients, treatment with lamivudine was started. Only one patient with occult HBV infection developed a viral reactivation that was controlled by the addition of adefovir to lamivudine. No hepatic adverse events were reported in this cohort of patients.

Of the 85 patients assessed, seven were positive for hepatitis C virus (HCV) serology and HCV-RNA documented an active infection in three cases. Patients were treated without the occurrence of significant adverse events; in one case, alemtuzumab administration was temporarily interrupted due to a significant increase of HCV-RNA associated with grade 1 alteration of liver enzymes.

Discussion

The present retrospective study evaluated the routine clinical use of low-dose alemtuzumab in 11 Italian haematological centres. The main purpose was to assess the efficacy and safety of this treatment approach in the 'real world' population,

Table IV. Hepatits B and C virus during alemtuzumab.

	no.	%
Hepatitis B virus (HBV)	83	100
HBV-exposed	14	17
Active (HBsAg positive)	5	6
Occult (HBcAb positive)	9	11
Hepatitis C virus (HCV)	85	100
HCVAb positive	7	8
HCV-RNA positive	3	4

HBsAg, Hepatitis B surface antigen; HBcAb, anti-hepatitis B core antigen; HCVAb, anti-HCV.

where therapies investigated in clinical trials are actually used (Gribben, 2010; Danese *et al*, 2011). By means of a similar approach, an Austrian retrospective study confirmed the activity of standard dose alemtuzumab in an unselected cohort of pretreated CLL patients in the routine clinical practice setting (Fiegl *et al*, 2006, 2010); however, the reported severe infection rate was 37%, confirming the toxicity of this treatment when given at full dose (Fiegl *et al*, 2006).

Demographic data of the 108 patients analysed in the present study show that low-dose alemtuzumab was preferentially used to treat older patients. In addition, the majority of the patient population was highly pretreated and in many cases patients also carried adverse clinical and biological prognostic factors. Specifically, there was a high prevalence of del(17p) and nearly half of the patients were treatment-refractory. The baseline assessment revealed that patients typically presented with progressive lymphocytosis and/or marrow insufficiency, with only a minor proportion of patients showing bulky lymph nodes or spleen. The decision to use alemtuzumab at lower doses appears to be the net result of the will to offer an effective treatment and the consideration of the frailty of the patients, in terms of age, comorbidities and previous exposure to highly toxic regimens.

All patients were treated according to a defined low-dose alemtuzumab schedule (i.e. total weekly dose ≤45 mg, cumulative dose ≤600 mg, treatment duration ≤18 weeks) and most of them completed the planned treatment. Despite this, patients were treated quite heterogeneously and none of the applied schedules appeared superior; however, due to the variability of alemtuzumab schedule and the retrospective nature of the study, it is not possible to draw definitive conclusions. In this regard, we suggest that schedules validated in Phase II trials should be used (Cortelezzi *et al*, 2009; Gritti *et al*, 2011).

The concomitant use of alemtuzumab and corticosteroids provided interesting results in high risk CLL and became part of the common practice in many institutions (Pettitt *et al*, 2006). Only three patients received a continuous dose of corticosteroids during low-dose alemtuzumab therapy, showing that this concomitant treatment is not common practice in Italy.

It can, however, be concluded that low-dose alemtuzumab appears to be a safe and effective treatment not only within clinical trials (Cortelezzi *et al*, 2005, 2009; Laurenti *et al*, 2005; Bezares *et al*, 2011; Gritti *et al*, 2011), but also in the routine clinical practice. Data from the present study and from previously published Phase II trials appear to be comparable in terms of efficacy to alemtuzumab given at conventional doses (Keating *et al*, 2002; Rai *et al*, 2002; Stilgenbauer & Dohner, 2002; Fiegl *et al*, 2006, 2010; Stilgenbauer *et al*, 2009). This favourable therapeutic effect is associated with an apparently better toxicity profile.

The main criticism of retrospective studies is that the interpretation of the data may be biassed by patient selection. In this regard, we believe that our cohort of patients is representative of the true population of patients treated with low-dose alemtuzumab in common practice. All the participating centres in this study adopted the policy to treat patients only with low-dose alemtuzumab due to safety concerns with the standard dose; therefore, there was no selection of standard versus low dose alemtuzumab. Additionally, the fairly low rate of early treatment interruption in our series (4%) is in line with previously published results of prospective trials (Cortelezzi *et al*, 2009; Gritti *et al*, 2011).

The only selection of patients in the study was relative to its clinical practice based nature. In fact, alemtuzumab is generally used on the base of previously published results clearly showing that the drug is almost ineffective or too toxic in patients with bulky disease and/or poor performance status (Keating *et al*, 2004; Osterborg *et al*, 2009). Accordingly, in our cohort there was a limited presence of patients with bulky adenopathies, significantly enlarged spleen and poor performance status that may not be representative of a highly pretreated and high risk CLL population. This selection should however not be considered a bias as it mirrors the common use of alemtuzumab in the clinical practice.

Treatment with alemtuzumab abolished the adverse effect of several prognostic factors; in particular, patients carrying del (17p) showed similar response rates with a non-significant trend towards a lower OS. Interestingly, the presence of del(11q) affected the response rates independently of other associated variables, particularly the presence of bulky lymph nodes, but this effect did not translate into differences in PFS or OS. The only variables associated with poor prognosis in terms of OS were treatment refractoriness (fludarabine-refractory) and/or the presence of bulky disease. While the first group of patients still benefited, at least in part, from alemtuzumab therapy (Keating *et al*, 2002; Stilgenbauer *et al*, 2009), an effective treatment of refractory patients with bulky disease is still lacking. Patients with ECOG PS two included in our study were characterized by both bulky disease and refractoriness to fludarabine and the prognosis was accordingly poor.

The toxicity of low-dose alemtuzumab was limited, confirming the good tolerability of treatment outside clinical trials (Cortelezzi *et al*, 2009; Gritti *et al*, 2011). In particular, the low rate of infections makes this treatment strategy suitable for frail and elderly patients.

Given that HBV and HCV infections are frequent in the Italian elderly population (Pendino *et al*, 2005), treatment with highly immunosuppressive drugs such as alemtuzumab may represent a major concern to clinicians. In the present study, patients with both active and occult HBV and active HCV infections received an adequate prophylactic treatment, with a careful monitoring of hepatic function and viral load, and they could be treated with low-dose alemtuzumab without the occurrence of serious adverse events.

In conclusion, the present study confirms that alemtuzumab given at low doses provides substantial clinical benefits to relapsed/refractory CLL patients with acceptable toxicity, thus proving the feasibility of this treatment approach in the current clinical practice.

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A.Co., R.F., A.Cu., G.G., F.R.M., L.L. and A.P. designed the research study. G.G., L.L., A.Cu., S.C., N.D.R., P.M., F.R.M., N.C., L.F., F.Z., V.C., F.M., S.M., A.P. and G.R. performed the research. A.Co., G.G. and A.P. analysed the data. A.Co., G.G. and R.F. wrote the paper. All the authors approved the final version.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig S1. (A) Overall survival and (B) progression-free survival according to age; age <70 years: black line (n = 63), age ≥70 years: red line (n = 45).

Table SI. Univariate analysis for response.

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